Plant Breeding **126**, 195—200 (2007)

doi:10.1111/j.1439-0523.2007.01294.x

Genotypic and environmental effects on selenium concentration of broccoli heads grown without supplemental selenium fertilizer

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With 5 tables

Received April 7, 2006/Accepted July 29, 2006
Communicated by W. E. Weber

Abstract

Selenium (Se) is an important trace element in human nutrition that is essential to normal health. Broccoli is known to accumulate relatively high concentrations of Se, and there is strong evidence that consumption of Se-enriched broccoli florets decreases cancer risk. In light of the above, this study was conducted to evaluate differences in Se concentration per head and total Se head content for a collection of broccoli hybrids (20) and inbreds (15) grown in field environments without supplemental Se fertilization. Our objectives were to assess the relative importance of genotype vs. environment in affecting Se levels and to determine if Se content is associated with other important horticultural traits. When analysed over three environments, there was a significant genotype effect for Se head concentration with hybrids, but not inbreds, but the environmental effect was about 10 times larger than that for genotype. Total Se content (ng/head) varied significantly among hybrids and inbreds, but as with concentration, environmental effects were also much larger for this trait. Head Se concentrations for hybrids ranged from 52.7 to 84.7 ng/g and total Se accumulation ranged from 563 to 885 ng/head. The same respective traits ranged from 49.3 to 80.0 ng/g and 678 to 876 ng/head for inbreds. There was no correlation between Se head concentration and head dry mass or days from transplant to maturity for either hybrids or inbreds. There was no evidence that Se might be diluted in broccoli heads as mass increases with cultivars that produce dense heads. Results indicate that it should be feasible to combine relatively high Se concentration or content with high head dry matter (DM), a phenotype that broccoli breeders might strive to achieve.

Key words: *Brassica oleracea* — Italica Group — doubled haploid — chemoprotection

Selenium (Se) is an essential human trace element needed for the activity of several important proteins including proteins with antioxidant activity, a protein involved in thyroid hormone metabolism and a protein associated with sperm maturation (Allan et al. 1999, Combs 2001). The Institute of Medicine has recommended that healthy adults consume at least 55 μg Se/day to prevent possible Se deficiency problems (National Academy of Science 2001). Over the past decade considerable evidence has advanced the hypothesis that intakes of Se well above 55 μg/day may be beneficial to human health, especially for the reduction of cancer. A randomized, placebo-controlled clinical trail demonstrated that supplementation of healthy men and women with 200 μg Se/day caused a dramatic decrease in total cancer incidence and mortality and specific decreases in lung and colorectal cancer (Duffield-Lillico et al. 2002).

The precise mechanism by which Se inhibits cancer is not known, but *in vitro* studies suggest that the active moiety is the methyl selenol metabolite produced when a molecule of Se enters the excretory pathway (Ganther 2001). Methyl selenol may be formed by methylation of the reduced selenide form of Se, or alternatively it may be generated by cleavage of the Se-C bond in the compound Se-methyl selenocysteine (SeMSC). Broccoli (*Brassica oleracea* L., Italica Group) contains Se in the form of SeMSC (Finley and Davis 2001). When consumed by mammals, SeMSC does not increase plasma Se concentrations, replenish Se in tissues, or restore glutathionine peroxidase activity and other forms of Se. On the contrary, it is more readily excreted into the urine (Finley 1998, 1999, Finley and Davis 2001). Based on the above results, Se from broccoli is deemed to have poor bioavailability. However, Se-enriched broccoli was more effective than selenate, selenite and selenomethionine (SeMet) in inhibiting preneoplastic lesions in the colons of rats (Finley and Davis 2001), reducing intestinal tumorigenesis in rats and mice (Finley et al. 2000, Davis et al. 2002), and reducing mammary tumours and activating pro-apoptotic genes in rats (Finley et al. 2001). The above evidence strongly indicates that Se from Se-enriched broccoli florets decreases cancer risk.

Though it has been demonstrated that high-Se broccoli can confer anticarcinogenic properties, conventionally grown broccoli does not typically contain the high levels of Se expressed by the broccoli used in the aforementioned cancer test studies. Typically, the high-Se broccoli utilized in such tests is produced by applying supplemental Se fertilizer to a crop in the field or by using a Se-enriched nutrient solution to produce broccoli grown in solid media or hydroponics. Broccoli has been used as a crop in remediation schemes involving irrigation with Se-contaminated waters (Banuelos 2002, Banuelos et al. 2003). However, large-scale field production of high-Se broccoli is problematic because Se salts have toxic properties and may not be safe to use as fertilizers.

An alternative means to facilitate production of high-Se broccoli might be to select varieties that accumulate enhanced levels above those typically observed. Banuelos et al. (2003) evaluated Se concentrations among four commercial broccoli hybrids receiving variable amounts of Se in contaminated water and did observe differences when high levels of Se were applied. However, conclusions drawn from this study are limited by the relatively small sample of broccoli cultivars and conditions of the experiment. Based on previous study

(Farnham et al. 2000), we know that different broccoli varieties can demonstrate significantly different concentrations of other minerals-like calcium (Ca) and magnesium (Mg). Although the environment can have a strong influence on the level of these minerals taken up from the soil, genetic control is also significant. In addition, Farnham et al. (2000) found the genetic component to be more important for inbreds (homozygotes) than for hybrids (heterozygotes), both for Ca and Mg.

In light of what is known about other minerals, we postulated that broccoli varieties would likely differ in Se concentration and that such differences could be under genetic control. Thus, the objectives of this study were to evaluate differences in Se concentrations and total vegetable content for a collection of broccoli inbreds and hybrids grown in field environments, to assess the relative importance of genotype vs. environment in affecting Se concentration, and to determine if Se concentration is associated with other important horticultural traits. Our approach was to assess differences in broccoli grown in conventional environments without supplemental Se fertilization.

Materials and Methods

Plant materials: Twenty commercial hybrid cultivars of broccoli (*B. oleracea* L., Italica Group) were selected for evaluation in field trials. This pool of hybrids was selected to represent a diverse phenotypic and genotypic array of commercial broccoli grown in the United States at the time of the trials, and included the following: 'Green Valiant', 'Arcadia', 'Greenbelt', 'Sultan' and 'Marathon' from Sakata Inc. (Salinas, CA, USA); 'Liberty', 'Viking', 'Captain', 'Major', 'Pirate' and 'Packman' from Peto Seed Co. (Saticoy, CA, USA); 'Sabre' and 'Baron' from Asgrow Seed Co. (San Juan Bautista, CA, USA); 'Everest' from Syngenta Seed Co. (Golden Valley, MN, USA); 'Headline', 'Zeus' and 'Charade' from Takii Seed (Salinas, CA, USA); 'Eureka' from Stokes (Buffalo, NY, USA); 'Excelsior' from Harris Moran (Modesto, CA, USA) and 'Claudia' from Ferry Morse (San Juan Bautista, CA, USA). In addition to these 20 varieties, 15 inbred (doubled haploid) lines developed at the U.S. Vegetable Laboratory in Charleston, SC were evaluated. These inbreds were developed using standard techniques of anther culture (Farnham 1998). Inbred designations and their parental source are as follows: USVL013, USVL020 and USVL071 derived from 'Everest'; USVL021, USVL022, USVL028 and USVL070 from 'Futura' (Asgrow Seed Co.); USVL030 from 'Green Valiant'; USVL036, USVL042 and USVL045 from 'High Sierra'; USVL049 and USVL073 from 'Marathon'; USVL062 from 'Sultan' and USVL074 from 'Viking'. All inbred seed were produced onsite at the U.S. Vegetable Laboratory. This pool of inbreds was also selected to represent a diverse phenotypic and genotypic array.

Materials evaluated in this study were originally generated in research to assay Ca and Mg concentration of broccoli vegetable heads (Farnham et al. 2000). On completion of that original work, all dried plant tissue samples were stored and maintained for future use. Considering the emerging interest in Se, we deemed the previously generated samples to be very suitable to serve as the needed experimental materials to test our objectives related to Se stated above.

Plant culture: In 1996, separate trials were conducted for hybrids and inbreds because of limited inbred seed supplies. The trials were grown in adjacent rows of the same field. Hybrids and inbreds were seeded into trays in a greenhouse in Charleston during the first and second weeks of August, respectively. On 28 August, hybrid seedlings were transplanted to the field, and inbreds were transplanted on 7 September. Both hybrids and inbreds were grown in a randomized complete block design, with three replications for hybrids and two for inbreds. Hybrid plots contained a single row of 20 plants while inbred plots contained six to 10 plants. The trials were grown according to standard cultural practices for the region (Cook and Ezell 1983) and were identical for both trials. Additional field preparation and spacing were as described by Farnham et al. (2000). When conditions became dry, natural rainfall was supplemented by overhead irrigation.

In 1997 and 1998, very similar trials were conducted. In each experiment, hybrids and inbreds were seeded to the greenhouse during the first week of August, and all entries were transplanted to the field on 27 August 1997 and 4 September 1998. Both hybrids and inbreds were grown in the same randomized complete block design with three replications each. Spacing, cultural practices, fertilization and irrigation were the same as the previous year.

Three different fields on the same farm were used over the 3 years. Following the 1996 and 1997 growing seasons, all fields on the farm received approximately 2.24 metric tons of dolomitic limestone per hectare. All three fields had the same soil classification of Yonges loamy sand (fine loamy mixed, thermic Albaqualfs). During each trial, soil samples were taken at random from each field and standard soil tests were conducted on each. Soil Se was also measured by conducting a standard acid digestion [Method 3050B of U.S. EPA (1982) and Kimbrough and Wakakuwa (1989)] of the soil followed by atomic absorption (furnace type) analysis of digests using standard Se method 7740 of U.S. EPA (1982), employing a SpectrAA 220 Zeeman instrument (Varian, Palo Alto, CA, USA). Rainfall amounts during the duration of each trial were recorded for each field.

Head harvest: As plants approached maturity, plots were evaluated every 2–3 days until individual plots matured. Both hybrid and inbred heads were considered mature and ready to harvest when head diameter reached 10–12 cm, and sampling of all entries was underwent when heads attained this size range. With hybrids, four random heads per plot were collected, cut to a 10 cm length, and bulked for subsequent Se analysis, and with inbreds, three heads were collected and bulked. Sampling date of heads for each hybrid or inbred entry was recorded to determine the mean number of days from transplanting to harvest stage (DTH).

Sample preparation and mineral analysis: All heads harvested for mineral analysis were placed in paper bags and dried at 60°C. When samples reached a constant dry weight, weights were recorded to determine average head dry mass (DM) per plant. Dried heads were ground to a 0.5-mm sieve size using a cutting mill (Model SM1; Brinkmann Instruments, Westbury, NY, USA) to homogenize each sample. Approximately 0.5 g of dried broccoli was weighed out on a four-place balance in a 100 ml beaker. Batches were set up with three blanks and three standards of NBS 1567a Wheat Flour (National Bureau of Standards, Gaithersburg, MD, USA). The following were added to the samples, blanks and standards: 10 ml of 40% magnesium nitrate (Alfa Aesar, Ward Hill, MD, USA), 10 ml of 16 M nitric acid and 2 ml of 6 M hydrochloric acid (J. T. Baker, Phillipsburg, NJ, USA). The samples were covered with watch glasses, placed on a hot plate, and refluxed on low heat setting for 24 h. After 24 h, watch glasses were removed and samples were heated to dryness. Samples were then placed in a muffle oven at 490°C for 14 h. Samples were then dissolved in 10 ml of 12 M hydrochloric acid and diluted to 25 ml with deionized water for analysis. Samples were analysed with a Perkin Elmer 5100PC AAS FIAS with a hydride generator (Perkin Elmer, Wellesley, MA, USA). Magnesium nitrate is included as a matrix modifier in this procedure to prevent Se from volatilizing during wet and dry digestion steps, and this method results in an approximate 100% recovery of Se from plant material (Finley et al. 1996).

Statistical analysis: Hybrids and inbreds were analysed separately. The three-hybrid trials were first analysed as separate experiments to examine entry effects on Se concentrations and other traits (e.g. total Se yield, mean head DM) in each environment. The data from the three trials were then combined to examine environment and genotype by environment interaction. The inbred trials were analysed in the same manner. Analysis of variance was performed using Proc GLM of SAS (release 6.12, SAS Institute, Inc., Cary, NC, USA). Pearson's correlation coefficients were calculated for all pairwise comparisons between mean head weight, mean DTH, head Se concentration and head Se yield for inbred and hybrid entries.

Results

When analysed across all three environments, a majority of the variation observed for Se head concentration, Se head content and head DM could be attributed to the environment (Table 1). Only DTH exhibited more variation because of genotype than environment. The above observations were equally true for hybrids and inbreds (Table 1). Averaged across all genotypes, Se head concentration and total Se content increased almost twofold (e.g. from 50 to 95 ng/g) for hybrids from 1996 to 1998 and more than twofold (e.g. from 34 to 89 ng/g) for inbreds from 1996 to 1998 (Table 2). This trend was even more accentuated for total Se content per head that was almost three times greater in 1998 compared with 1996 for both hybrids and inbreds.

Selenium concentrations of soils sampled from the three trials were very similar (e.g. all were approximately 0.50 mg/kg). Soil concentrations of most plant nutrients were generally rated high (data not shown), indicating that no mineral was limiting growth in any trial. Soil pH was the one condition that changed consistently over 3 years starting at 5.3 in 1996 and increasing to 5.8 in 1997 and 6.3 in 1998. Total growing season rainfall and distribution were very similar at 31.0 and 36.2 cm in 1996 and 1998, respectively. Rainfall was much higher at 59.5 cm during the 1997 growing season with the majority of the increase coming from the first month of growth in September.

When all environments were considered, there was a significant genotypic effect on Se concentration (hybrids only) and total Se content, as well as head DM and DTH (Table 1), although the genotypic effects for the Se traits were not as dramatic as effects because of environment. When each environment was analysed separately, the genotypic effect was only significant for Se concentration in 1996 with inbreds and in 1998 with hybrids (data not shown). For total Se content, inbreds exhibited significant genotypic differences in 1996 and 1998 but hybrids only showed differences in 1998 (data not shown). Contrary to Se traits, both head DM and DTH exhibited significant genotypic effects in all three environments combined (Table 1) as well as individually (data not shown). Genotype-by-environment effects were present for DM and DTH, but these interactive effects were absent for Se concentration and only present in hybrids for total head Se (Table 1).

Averaged across environments, Se concentration of hybrids ranged from a low of 52.7 ng/g for 'Major' to a high of 84.7 ng/g for 'Sabre' (Table 3). The same two hybrids exhibited a respective low and high for total Se head content of 563 and 885 ng/head. The four hybrids with the highest Se concentrations also had the highest Se contents per head. Likewise, the four hybrids with the lowest Se concentrations also had the lowest total contents. With hybrids, Se concentration was significantly correlated with total Se content but DM was not. Mean head DM and DTH among hybrids also exhibited a significant positive correlation.

The range in Se concentrations among inbreds averaged across all environments was similar (e.g. 49.3–80.0 ng/g) to that observed for hybrids, although with inbreds this difference was not significant (Table 4). The range in total Se content among inbreds was 480–1231 ng/head, which is greater than that observed for hybrids. This difference was highly significant. Unlike hybrids, both Se concentration and head DM were significantly and positively correlated with total Se content (Table 5). Similar to hybrids, inbred head DM and DTH were also positively correlated.

Discussion

When the same samples examined for Se concentration and content herein were evaluated for Ca and Mg content previously (Farnham et al. 2000), environmental variation was also found to be highly significant for both hybrids and inbreds. It is to be expected that the level of any mineral that must be taken up from the soil by the roots will likely be highly influenced by the overall soil environmental conditions. In addition to significant environmental effects for Ca and Mg,

Table 1: Mean squares from the analysis of variance of Se concentration (conc.), Se head content, head DM and DTH for broccoli hybrid (20) and inbred (15) genotypes (G) grown in three environments (E)

		Mean squares			
Source	df	Se conc. (ng/g DM)[1]	Se content (ng/head)[1]	Head DM (g/head)[1]	DTH (days)[1]
Hybrids					
E	2	34 009.8**	10 683 180**	225.90**	317.5**
Rep (E)	6	3376.5**	681 577**	5.02*	30.5
G	19	819.7**	97 678*	10.03**	1135.3**
G × E	38	487.6	86 093*	6.01**	46.8**
Error	114	343.7	49 284	1.73	17.9
Total	179				
Inbreds					
E	2	28 295.0**	5 390 831**	154.98**	337.2**
Rep (E)	5	2857.5**	738 639**	14.06*	23.9
G	14	491.9	265 607**	43.97**	1601.4**
G × E	38	317.9	64 022	7.98*	108.0**
Error	114	387.3	65 454	4.53	12.9
Total	173				

[1]Se, selenium; DM, dry mass; DTH, days from transplant to harvest.
*,**Significant at P = 0.05 and P = 0.01, respectively.

Table 2: Yearly mean values for all genotypes for head Se concentration (conc.), Se head content, head DM and DTH for hybrid and inbred broccoli grown in 1996, 1997 and 1998

Year	Se conc. (ng/g DM)[1]	Se content (ng/head)[1]	Head DM (g/head)[1]	DTH (days)[1]
Hybrid				
1996	49.5	485	9.91	68.4
1997	60.1	634	10.56	70.8
1998	95.0	1279	13.55	73.0
Mean	68.2	799	11.34	70.7
Inbred				
1996	33.6	439	13.41	70.7
1997	56.6	552	9.73	72.9
1998	88.8	1121	12.65	76.8
Mean	62.7	737	11.77	73.8

[1]Se, selenium; DM, dry mass; DTH, days from transplant to harvest.

Table 3: Mean values for head Se concentration, total selenium content, head DM and DTH for hybrids across three test environments

Hybrid	Se conc. (ng/g DM)[1]	Se content (ng/head)[1]	Head DM (g/head)[1]	DTH (days)[1]
Sabre	84.7	885	10.15	73.8
Viking	81.9	913	10.62	79.3
Green Valiant	80.3	941	11.24	74.1
Everest	79.2	917	11.05	53.9
Arcadia	75.1	841	10.80	75.1
Zeus	75.0	832	9.97	58.2
Packman	72.6	805	10.76	55.9
Excelsior	71.7	820	10.97	77.2
Eureka	70.7	842	11.89	75.1
Marathon	69.1	883	13.44	85.3
Pirate	67.6	851	12.11	79.3
Headline	66.3	798	11.11	68.4
Claudia	65.4	812	11.78	61.2
Baron	63.6	727	10.78	67.0
Sultan	62.1	751	11.66	68.2
Charade	60.0	841	13.98	95.8
Greenbelt	56.4	674	11.63	79.4
Captain	55.3	579	10.15	57.1
Liberty	54.5	713	12.34	75.0
Major	52.7	563	10.35	54.9
Mean	68.2	780	11.34	70.7
$LSD_{0.05}$	17.3	207	1.23	3.9

[1]Se, selenium; DM, dry mass; DTH, days from transplant to harvest.

Table 4: Mean values for head Se concentration, total selenium content, head DM and DTH for inbreds across three test environments

Inbred	Se conc. (ng/g DM)	Se content (ng/head)	Head DM (g/head)[1]	DTH (days)[1]
USVL030	80.0	876	11.17	82.6
USVL070	74.7	882	11.20	54.3
USVL073	73.0	1231	17.36	95.7
USVL071	69.1	763	10.81	63.4
USVL045	68.1	854	12.10	71.8
USVL021	65.7	480	7.58	65.8
USVL028	61.6	515	8.65	72.5
USVL036	60.6	709	12.51	75.0
USVL042	59.1	808	13.63	95.9
USVL020	58.6	677	11.27	65.3
USVL022	56.2	620	10.70	63.3
USVL074	55.6	687	12.73	94.5
USVL013	55.5	640	11.35	50.1
USVL062	54.0	629	11.24	74.1
USVL049	49.3	678	14.30	82.8
Mean	62.7	737	11.77	73.8
$LSD_{0.05}$	19.6	255	2.12	3.6

[1]Se, selenium; DM, dry mass; DTH, days from transplant to harvest.

Table 5: Pearson's correlation coefficients for genotype means of head Se concentration (conc.), head selenium content, head DM and DTH averaged across three environments (correlations for hybrids are above the diagonal; inbreds are below)

	Se conc.	Se content	Head DM[1]	DTH[1]
Se Conc.	1	0.856**	−0.295	0.032
Se Content	0.592*	1	0.212	0.361
Head DM	−0.017	0.778**	1	0.701**
DTH	−0.019	0.410	0.621**	1

[1]Se, selenium; DM, dry mass; DTH, days from transplant to harvest.
*,**Significant at $P = 0.05$ or $P = 0.01$, respectively.

genotypic differences were always observed in every environment. Overall, variation among replicates was greater in this study for Se than it was for previous work with Ca and Mg (Farnham et al. 2000), and this likely created a much larger error variance that confounded the detection of genotypic differences. Thus, with Se it required the greater statistical power provided by evaluating multiple environments to detect genotypic differences.

One environmental factor of this study that probably accounts for increased levels of Se (expressed as concentration or total content) in broccoli heads from 1996 through 1998 is soil pH. Numerous other studies (Cary and Allaway 1969, Gissel-Nielsen 1971, Gupta and Winter 1975) evaluating species other than broccoli, have shown that plant accumulation of Se increases as pH rises from acid to near neutral. No other environmental component accounted for in these trials would readily explain the significant differences in Se content that were observed among environments. For example, levels of Se in the respective trial soils were indistinguishable at 0.50 mg/kg. These soil levels are very much within the range (0.1–3.9 mg/kg) reported as background levels in Eastern United States soils (New York State Department of Environmental Conservation 2001). Additional research with broccoli might examine liming as a potential means to optimize the environment for maximum Se uptake. The general absence of genotype by environment interactions observed for Se traits in this trial indicates that increasing pH would probably enhance uptake of Se by all genotypes, and not one over another.

Selenium content is a product of Se concentration and total DM per head, and thus, one would expect both of the latter to be correlated with content. However, with hybrids, only Se concentration was significantly correlated with content while DM was not (Table 5). This result indicates that Se concentration is likely the more important factor determining the total content of Se found in a head of hybrid broccoli. A positive correlation between mean head DM and DTH among hybrids observed in this study is largely explained by the fact that relatively early maturity hybrids-like 'Captain' and 'Major' have less dense heads (i.e. heads with lower DM) than later maturity hybrids-like 'Marathon' and 'Liberty'.

Although studies characterizing nutritional or health-promoting constituents of broccoli typically focus on the concentration of the respective constituent, we argued previously

(Farnham et al. 2004) that the total content of a constituent per broccoli head may be as or more important than concentration. The logic for this stems from the fact that in many markets, broccoli is sold as a single head or bunch of heads rather than by the pound and that consumers are likely to eat a whole head of broccoli at one time rather than eat a given weight. This being probable, then the content per head becomes the amount of the constituent often eaten during a meal.

For both inbreds and hybrids, total Se content exhibited small but significant genotypic differences across three environments while the genotype effect for Se concentration was only significant for hybrids. This may lend support to the possibility that selecting genotypes with enhanced Se based on total content per head might be possible while selection based only on concentration is much less likely to be effective. As was observed for other minerals in broccoli (Farnham et al. 2000), the deposition of Se in broccoli heads appears to be different for hybrids, relative to inbreds. Nearly 100% of broccoli consumed in North America and Europe is harvested from hybrids. Thus, Se enhancement in these types of varieties will provide more potential benefit to consumers. However, broccoli breeders conduct much of the plant breeding process, including making most selections, during the inbreeding phase. Thus, it will be important to recognize the likelihood of different accumulation dynamics in hybrids, in order to create the best new varieties.

An important observation from this study is that Se concentration was not correlated with head DM (Table 5). Farnham et al. (2000) found that Ca and Mg concentrations in broccoli hybrids and inbreds were often negatively correlated with head DM. In general, with Ca and Mg, the above authors observed an apparent dilution effect with lower concentrations accompanying increased accumulations in head density and DM. This phenomenon is particularly undesirable considering that the newest broccoli hybrids that garner significant market share mature later and accumulate increased head DM compared with older hybrids. Consequently, these hybrids can exhibit lower mineral concentrations. With Se, there is no indication of a similar dilution effect evident in our results. This indicates that it should be feasible to identify broccoli hybrids that have relatively high Se concentration or content combined with high head DM, a likely phenotype that breeders would strive to achieve. In our study, Se content was actually positively correlated with head DM, although only for the inbred lines (Table 5).

Head Se concentrations reported herein are much lower than those observed in other trials wherein broccoli was fertilized with Se salts (Finley and Davis 2001) or watered with effluent (Banuelos 2002) or contaminated water (Banuelos et al. 2003) containing high Se concentrations. In the above cases, concentrations of Se in heads were at least 100 times greater than in the present study. However, the levels we report herein are similar to those measured in other broccoli samples harvested from fields with no supplemental Se and having average Se concentration and availability (Finley et al. 1996). It will be useful for future studies to determine whether Se concentration or content rankings of a diverse set of genotypes, as described herein, will vary from the present results, when that set is grown in an environment supplying higher than normal levels of the mineral. It will be important to study this question before the likelihood of success in breeding for enhanced Se content can be fully assessed.

Acknowledgements

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